THE PROTECTION OF PARASITES IN THE DIGESTIVE TRACT AGAINST THE ACTION OF THE DIGESTIVE ENZYMES

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In carrying out an investigation on the rate of oxidation of enzymes and their corresponding pro-enzymes it has been necessary to make use of many dogs for collecting pancreatic juice and preparing secretin and enterokinase. Tapeworms and roundworms were found in the intestines of many of these animals and the question so often raised presented itself, viz., why is it that these worms are not digested by the trypsin? Various explanations have been offered in answer to this question, such as the existence of an antisubstance in the worm which inhibits the action of the trypsin, or the resistance of the cuticle to the activity of the enzyme or the fact that the parasites are alive, etc. Lillie and others have shown that the mucosa of the stomach and intestine possesses intense oxidative properties and Burge has found that trypsin, in common with all the digestive enzymes, is relatively easy to oxidize. On the basis of these two facts we have advanced an hypothesis according to which the mucosa of the digestive tract by means of the oxidative processes going on in its cells is able to maintain its integrity during life by rendering inactive the enzyme solution immediately in contact with it. This assumes that there are two opposing activities at work, viz., the active enzyme within the lumen of the digestive tract attempting as it were to digest the cells of the mucosa, while the oxidative processes of these cells are rendering the enzyme inactive and hence protecting the mucosa from digestion. The same explanation might be given for the fact that parasites are able to live in the intestinal juices without being digested. The assumption is that the oxidative processes going on in the cells of the parasite exposed to the action of the trypsin are oxidizing the enzyme. Thus the parasite, like the mucosa of the tract itself, is protected from digestion.

The following experiments and observations were made to determine if any experimental evidence could be brought forward in support of this assumption:

A dog was etherized and killed with chloroform. The intestine of this animal was slit open and the tapeworms and roundworms removed. The duodenum and several inches of the intestine following were thoroughly washed and the mucosa gently scraped with the handle of a scalpel. Enterokinase was prepared by extracting this scraping with 0.7 per cent. sodium chlorid. The

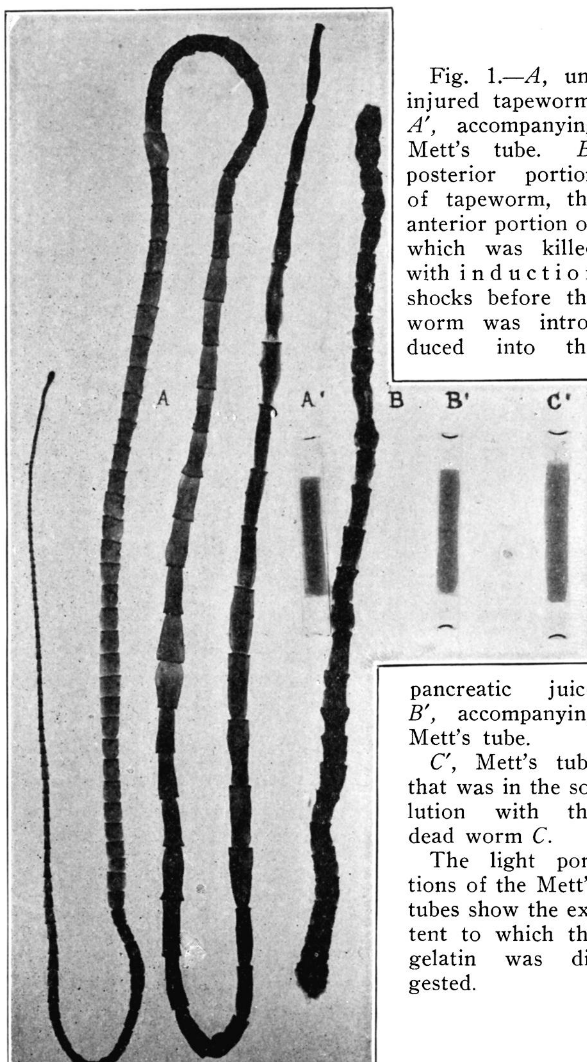

Fig. 1.—*A*, uninjured tapeworm. *A'*, accompanying Mett's tube. *B*, posterior portion of tapeworm, the anterior portion of which was killed with induction shocks before the worm was introduced into the pancreatic juice. *B'*, accompanying Mett's tube. *C'*, Mett's tube that was in the solution with the dead worm *C*. The light portions of the Mett's tubes show the extent to which the gelatin was digested.

secretin was prepared by extracting the hashed mucosa with 200 c.c. of 0.4 per cent. hydrochloric acid. This extract was boiled and neutralized while boiling with 1 per cent. sodium hydroxid. On filtering a perfectly clear solution was obtained. This secretin was injected into the jugular vein of an etherized dog and approximately 100 c.c. of clear pancreatic juice were obtained from the pancreatic duct.

Thirty c.c. of this pancreatic juice were activated by the addition of 3 c.c. of enterokinase. This trypsin solution was sterilized by exposing it for five minutes to the radiation from a 2,400 candle power quartz mercury vapor burner at a distance of 12 cm. A tapeworm, *Taenia serrata,* about 40 cm. in length, was washed in tap water, rinsed in distilled water, and introduced into 10 c.c. of the solution. Another tapeworm of about the same size was selected and one electrode from the secondary of a large induction coil was placed near its anterior end while the other electrode was moved back and forth over the anterior half of the parasite until no response to stimulation was obtained from this portion. In this manner the anterior part of the tapeworm was killed without breaking the cuticle. A third worm of similar size was selected and the entire worm killed by means of induction shocks. These worms were placed in vessels containing 10 c.c. of the activated pancreatic juice. The three vessels containing the worms were allowed to stand at room temperature for twelve

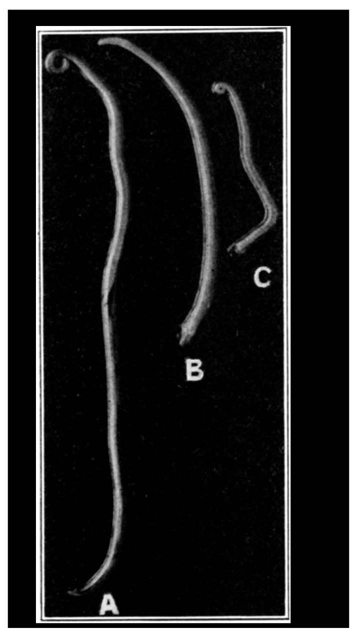

Fig. 2.—*A,* uninjured roundworm. *B* and *C,* undigested halves of two worms, the dead halves having been digested.

hours. At the end of this time the uninjured worm and the uninjured half of the other worm were stimulated by means of weak induction shocks. It was found that they were alive. These solutions were removed and 10 c.c. of fresh sterilized juice introduced into each vessel. At the end of the second twelve-hour period the anterior portion, which had been killed with induction shocks, as well as the entire worm which had been killed in the same manner had begun to be digested. After introducing into each vessel 10 c.c. of fresh juice the worms were permitted to stand for a third twelve-hour period. Thus the parasites were exposed to the action of the trypsin for thirty-six hours. Figure 1 shows the condition of the parasites at this time. *A* is the uninjured tapeworm; *A′*, a Mett's tube containing gelatin colored with Congo red which was placed simultaneously with the worm in the pancreatic juice in order to give an idea of the strength of the trypsin. The dark portion of the tube represents

the undigested gelatin and the light the empty tube from which the gelatin was digested. *B* is the worm the anterior part of which was killed with induction shocks, *B'*, its accompanying Mett's tube. *C'* is the tube which was in the solution with the dead worm *C*. It may be seen that no portion of the normal worm *A* was digested, that the dead portion of *B* was completely digested as was also the dead worm *C*.

Similar experiments were carried out using roundworms. Halves of roundworms were killed by means of induction shocks and these together with an uninjured worm were introduced into 15 c.c. of activated pancreatic juice, sterilized by ultra violet. The vessel containing the juice and the worms was placed in a thermostat at 38 C. At the end of ten hours the solution was replaced by 15 c.c. of fresh juice. At this time digestion of the dead portions had begun. The uninjured worm and the uninjured portions of the other two were still alive. At the end of the second ten-hour period the dead portions of the worms were almost completely digested. The juice was again changed and at the end of the third ten-hour period the uninjured portions were completely digested. A photograph of the worms was taken at this time (Figure 2); *A*, is the uninjured worm; *B* and *C*, the undigested halves of the two worms, the dead halves having been digested.

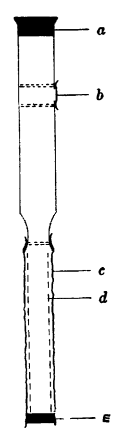

Fig. 3.—*a*, pipette closed with stopper; *b*, oneway valve; *c*, segment of round worm; *d*, platinum mesh; *e*, thin rubber and ligature.

The experiments now to be described were devised to show that the uninjured parts as well as the uninjured worms were not digested because of the protection afforded by the oxidative processes going on in them. Two segments, each 5 cm. in length, were cut from a roundworm. Into the lumen of one segment (Fig. 3, *c*) was introduced a cylindrical piece of platinum mesh (*d*) previously covered with platinum black. Around one end of the segment was wrapped a narrow strip of thin rubber (*e*) about which a ligature was tied thus closing this end of the segment. A pipette (*a*) was inserted into the open end. This was held in position by means of a ligature. The segment was filled with hydrogen peroxid through the pipette, the pipette stoppered and a one-way escape valve (*b*) arranged so that the oxygen liberated from the hydrogen peroxid by platinum black could not escape from the segment through the pipette until the pressure reached approximately 25 mm. of mercury. The pressure arising from the liberated oxygen forced it through the body wall of the roundworm. In this manner all parts of the roundworm were exposed to oxygen presumably in a nascent state. At this stage of the experiment the segment of the worm was dead and the only processes going on within it in

any way comparable to any of the living processes, was the artificial oxidative process. One end of the other segment cut from the same roundworm was ligatured and the segment filled with a solution made by decomposing hydrogen peroxid with platinum black. The other end was closed by a ligature and the preparation introduced into the activated pancreatic juice along with the other preparation. The vessel containing the preparations was placed in a thermostat at 38 C. and allowed to remain for forty-eight hours. At intervals of two hours fresh hydrogen peroxid was introduced into the segment into which the pipette was tied. At the end of twenty-four hours the pancreatic juice was removed from the vessel and fresh pancreatic juice added. At the end of forty-eight hours there was very little if any indication of digestion in the segment which was permeated by oxygen while the other segment was digested. The conclusion is drawn that nascent oxygen prevented the segment of the worm from being digested by rendering inactive the enzyme solution in contact with it.

SUMMARY

Tapeworms and roundworms from the intestine of the dog are not digested when introduced into activated pancreatic juice so long as they remain alive but are digested when dead. If any part of them be killed this part is digested.

A dead roundworm which is ordinarily digested when introduced into activated pancreatic juice, can be prevented from being digested by keeping the dead body wall constantly permeated with nascent oxygen.

The oxidative processes of the living parasites enable them to withstand the action of the digestive juices by oxidizing the enzyme solution immediately in contact with them.

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